

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)


DP Barcode: 425244

EPA MRID Number 49540301

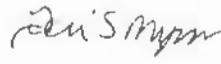
Data Requirement:
EPA DP Barcode D425244
EPA MRID 49540301
EPA Guideline OCSPP 850.1075

Test material: Biomist 3+15 ULV formulation
Common name: Permethrin TEP (High PBO: Permethrin Ratio)
Chemical name: IUPAC: Not Reported
CAS name: Not Reported
CAS No.: 52645-53-1 (permethrin); 51-03-6 (Piperonyl butoxide)
Synonyms: None Reported
Purity: 2.90% permethrin
14.29% Piperonyl butoxide

Primary Reviewer: John Marton, Ph.D.
Environmental Scientist, CDM Smith

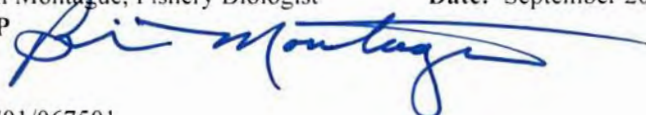
Signature: 
Date: 04/16/15

Secondary Reviewer: Teri S. Myers, Ph.D.
Environmental Scientist, CDM Smith

Signature: 
Date: 05/15/15

Primary Reviewer: Brian Montague, Fishery Biologist
ERB5/EFED/OPP/OCSPP

Date: September 2015



EPA PC Code 109701/067501

Date Evaluation Completed: September 21, 2015

CITATION: Brougher DS, Zhang L, Martin KH, Gallagher SP. 2014. Permethrin TEP (High PBO:Permethrin Ratio): A 96-Hour Flow-Through Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*). Unpublished study performed by Wildlife International, Evans Analytical Group, Easton, MD. Laboratory report number 701A-115. Study sponsored by Consumer Specialty Products Association, Washington, DC. Study completed December 29, 2014.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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PMRA Submission Number {.....}

EPA MRID Number 49540301

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, bluegill sunfish (*Lepomis macrochirus*) were exposed to a TEP blend containing Permethrin and Piperonyl butoxide (PBO) at nominal concentrations of 0 (negative and solvent controls), 13, 25, 50, 100, and 200 µg formulation/L under flow through conditions. This ratio of two actives is used in Biomist 3+15 ULV Mosquito Control product. The nominal concentration levels corresponded to nominal permethrin concentrations of 0.377, 0.725, 1.45, 2.90, and 5.80 µg ai/L and nominal concentrations of 1.86, 3.57, 7.15, 14.3, and 28.6 µg ai/L. The reviewer-calculated time-weighted average (TWA) concentrations for permethrin were 0.471, 0.916, 1.41, 3.18, and 7.58 µg ai/L, while the TWA concentrations for PBO were 1.86, 3.48, 6.40, 14.6, and 26.8 µg ai/L. The 96-hr LC₅₀ values for permethrin, PBO, and permethrin + PBO were 2.12, 9.67, and 11.8 µg ai/L, respectively. Sublethal effects included surfacing, lying on the bottom of the test vessel, and loss of equilibrium, and these effects were restricted to the two highest treatment levels. Based on the results of this study, Permethrin TEP (high PBO:Permethrin ratio) would be classified as very highly toxic to *Lepomis macrochirus* in accordance with the classification system of the U.S. EPA.

This study is sound and is classified as acceptable for use in Agency risk assessments where toxicity of this mixture product is applicable.

Results Synopsis

Test Organism Size: 0.26 (0.12-0.43) g; 2.8 (2.3-3.5) cm; based on 10 negative control fish

Test Type: Flow-through

Permethrin + Piperonyl butoxide 17.19 % (2 actives)

LC₅₀: 11.8 µg ai/L 95% C.I.: 9.85-14.0 µg ai/L

Probit Slope: 9.26 95% C.I.: 5.60-12.9

Permethrin:

LC₅₀: 2.12 µg ai/L 95% C.I.: 1.78-2.53 µg ai/L

Probit Slope: 9.35 95% C.I.: 5.70-13.0

Piperonyl butoxide:

LC₅₀: 9.67 µg ai/L 95% C.I.: 8.09-11.6 µg ai/L

Probit Slope: 9.19 95% C.I.: 5.55-12.8

Individual ingredient estimates based on a ratio of 2.90% Permethrin, 14.29% PBO

Endpoint(s) Affected: mortality, sub-lethal effects

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the OECD Guidelines for Testing of Chemicals, Guideline 203, *Fish, Acute Toxicity Test*; the U.S. EPA Series 850 Ecological Effects Test Guidelines, OCSPP 850.1075, *Fish Acute Toxicity Test, Freshwater and Marine*, and ASTM Standard E 729-96, *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians*.

The following deviations from OCSPP 850.1075 were noted:

1. Particulate matter and chlorine concentrations of the dilution water were not reported.
2. Initial fish weight was lower than recommended.
3. pH range was higher than recommended for this species
4. Nominal concentration ratio was 50% rather than 60% between concentration levels

This deviations did not impact the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NouSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999), with the following exceptions: periodic analyses of water for potential contaminants were not performed accord to GLP Standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods; and the characterization of the test substance and one of the reference substances and their stability under storage conditions were not performed according to GLP Standards.

A. MATERIALS:

1. Test material	Permethrin TEP (High PBO:Permethrin Ratio)
Description:	Liquid
Lot No./Batch No. :	1404220006
Purity:	2.90% Permethrin, 14.29% PBO
Stability of compound under test conditions:	Reviewer-calculated TWA concentrations yielded coefficients of variation of 10.6-39.5% and 3.5-21.4% for permethrin and PBO, respectively.
Storage conditions of test chemicals:	Initially stored under ambient conditions but then moved to <10°C.

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Physicochemical properties of Permethrin TEP.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

2. Test organism: EPA recommends a cold water species (preferably rainbow trout *Oncorhynchus mykiss*) and warm water species (preferably bluegill sunfish *Lepomis macrochirus*). OECD recommends choice of species at discretion of testing laboratory.

Species tested: Bluegill sunfish (*Lepomis macrochirus*)
Age at test initiation: Juveniles
Weight at study initiation: 0.26 (0.12-0.43) g; n = 10 control fish at test termination
EPA recommends: mean 0.5 - 5 g.
Length at study initiation: 2.8 (2.3-3.5) cm; n = 10 control fish at test termination
EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∇ 1.0 cm for bluegill and 5.0 ∇ 1.0 cm for rainbow trout
Source: Osage Catfisheries, Inc., Osage Beach, Missouri
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The study authors reported that results from a non-GLP range-finding study indicated that the LC₅₀ was approximately 100 µg formulation/L. No further details were provided.

b. Definitive Study

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Table 1: Experimental Parameters

Parameter/ Criteria	Details	Remarks
<p><u>Acclimation</u></p> <p>Period: <i>The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days.</i></p> <p>Conditions: (same as test or not)</p> <p>Feeding:</p> <p>Health: (any mortality observed) <i>Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.</i></p>	<p>At least 14 days</p> <p>Same as test</p> <p>During acclimation fish were fed a commercially-prepared diet supplied by Sera North America (Montgomeryville, PA), supplemented with brine shrimp nauplii (<i>Artemia</i> sp.) supplied by INVE Aquaculture (Salt Lake City, UT). Fish were not fed for at least two days prior to test initiation.</p> <p>No mortalities and no signs of disease or stress were observed during the two-week acclimation period.</p>	
<p>Duration of the test: <i>The recommended test duration is 96 hours.</i></p>	96 hours	
<p><u>Test condition</u></p> <p>Static/flow-through</p> <p>Type of dilution system - for flow-through method.</p> <p>Renewal rate for static renewal: <i>Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.</i></p>	<p>Flow-through</p> <p>Continuous-flow diluter system</p> <p>N/A</p>	Flow rate provided approximately 9 volume additions per day.
<p>Aeration, if any: <i>Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.</i></p>	None reported	

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Parameter/ Criteria	Details	Remarks
<u>Test vessels</u> Material: (glass/stainless steel) Size: Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm. Fill volume: Fill volume is usually 15-30 L of solution.	Teflon®-lined stainless steel aquaria 25 L 15 L	
Source of dilution water Quality: Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water. 850.1075 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1075.pdf) OECD permits dechlorinated tap water. Dilution water should be intensely aerated before the study.	On-site well water was passed through a sand-filter (25 µm), aerated with spray nozzles, filtered to 0.45 µm, then UV-sterilized.	Water was from sourced from well – dechlorination not a factor.

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Parameter/ Criteria	Details	Remarks
<p><u>Water parameters:</u> Hardness: <i>EPA recommends 40 - 48 mg/L as CaCO₃ (OECD recommends 10 - 250 mg/L)</i> pH: <i>EPA recommends 7.2 - 7.6; monthly range < 0.8; (OECD recommends pH 6.0 - 8.5)</i> Dissolved oxygen: <i>EPA recommends: flow-through: 60%; (OECD guideline recommends at least 80% saturation value).</i> Temperature: <i>EPA: 17 or 22 °C for warmwater species, OECD recommends 21 - 25°C for bluegill</i> Total Organic carbon Particulate Matter Metals Pesticides Chlorine Intervals of water quality measurement : <i>Water quality should be measured at beginning of test and every 48 hours</i></p>	<p>Hardness: 132 mg/L as CaCO₃ pH: 8.0-8.1 D.O.: 7.1-8.7 mg/L (≥82% of saturation) Temperature: 21.88-22.01°C TOC: <1 mg/L Particulates: Not Reported Metals: See Reviewer's Comments Pesticides: None Detected Chlorine: Not Reported</p>	<p>Specific conductance: 334 µS/cm Alkalinity: 182 mg/L as CaCO₃ Temperature was measured in each test vessel at 0, 24, 48, and 96 hours. Temperature was also continuously measured in one negative control test chamber. Dissolved oxygen and pH were measured in one replicate of each control and treatment test chamber at test initiation and daily thereafter, with measurements alternating between replicates.</p>
<p><u>Number of replicates/groups:</u> <i>Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.</i></p>	<p>Controls; 2 Solvent Controls: 2 Treatments: 2</p>	<p>Nominal concentrations were approximately 50% of the next highest concentration</p>
<p><u>Number of organisms per replicate /groups:</u> <i>Number of organisms per replicate should be 10/concentration; OECD guideline recommends at least 7 fish/concentration.</i></p>	<p><u>Fish/concentration</u> control: 10/replicate solvent control: 10/replicate treated groups: 10/replicate</p>	

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Parameter/ Criteria	Details	Remarks
Biomass loading rate: <i>Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through</i>	Flow through Study 0.018 g/L in a 24-hour period 0.17 g/L at any given time	
Test concentrations: nominal: measured:	Formulation Nominal: 0 (negative and solvent controls), 13, 25, 50, 100, 200 µg formulation/L Permethrin: PBO Ratio: 1:4.5, 1:3.7, 1:4.6, 1:4.7, 1:3.5 Permethrin: Nominal: 0.377, 0.725, 1.45, 2.90, 5.80 Measured: 0.434, 0.956, 1.48, 3.12, 7.58 µg ai/L PBO: Nominal: 1.86, 3.57, 7.15, 14.3, 28.6 µg ai/L Measured: 1.95, 3.5, 6.76, 14.6, 26.8 µg ai/L	Reviewer-calculated TWA: Permethrin: 0.471, 0.916, 1.41, 3.18, 7.58 µg ai/L PBO: 1.86, 3.48, 6.40, 14.6, 26.8 µg ai/L Permethrin: PBO Ratio: 1:4.0, 1:3.8, 1:4.5, 1:4.6, 1:3.5
Solvent (type, percentage, if used): <i>The solvent should not exceed 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.</i>	DMF (0.1 mL/L)	
Lighting: <i>The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.</i>	16L:8D with a 30-minute low-light transition period	
Feeding: <i>Fish should not feed during the study.</i>	Fish were not fed during the definitive test	
Recovery of chemical Frequency of determination Level of quantization Level of detection	0, 48, 96 hours 0.240 µg ai/L- Permethrin 1.20 µg ai/L- PBO Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Parameter/ Criteria	Details	Remarks
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter/Criteria	Details	Remarks
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Sub-lethal effects	
Observation intervals : <i>Observation intervals should be a minimum of every 24 hours.</i>	4.5, 24, 48, 72, 96 hours	Observation intervals were correct
Were raw data included?	Yes	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Throughout the test, no mortalities occurred in the controls or mean-measured 0.434 and 0.956 µg permethrin/L treatment groups. Mortality was first observed after 96 hours in the top two doses, and by test termination, mortality was 5, 95, and 100% in the mean-measured 1.48, 3.12, and 7.58 µg permethrin/L treatment groups, respectively. The study authors reported an LC₅₀, based on mean-measured permethrin concentrations of 2.15 (1.83-2.53) µg permethrin/L.

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Table 3: Effect of Permethrin TEP on Mortality of *Lepomis macrochirus*.

Mean-Measured and (Nominal) Permethrin Concentrations µg ai/L/replicate	No. of fish at start of study	Observation Period					
		24 Hours		48 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative Control A	10	0	0	0	0	0	0
Negative Control B	10	0	0	0	0	0	0
Solvent Control A	10	0	0	0	0	0	0
Solvent Control B	10	0	0	0	0	0	0
0.434 (0.377) A	10	0	0	0	0	0	0
0.434 (0.377) B	10	0	0	0	0	0	0
0.956 (0.725) A	10	0	0	0	0	0	0
0.956 (0.725) B	10	0	0	0	0	0	0
1.48 (1.45) A	10	0	0	0	0	1	10
1.48 (1.45) B	10	0	0	0	0	0	0
3.12 (2.90) A	10	0	0	1	10	10	100
3.12 (2.90) B	10	0	0	0	0	9	90
7.58 (5.80) A	10	0	0	7	70	10	100
7.58 (5.80) B	10	0	0	5	50	10	100
LC ₅₀	2.15 (1.83-2.53)						
Positive control	Not used						
0.434 (0.377 µg ai/L) = 13 µg formulation/L nominal 0.956 (0.725 µg ai/L) = 25 µg formulation/L nominal 1.48 (1.45 µg ai/L) = 50 µg formulation/L nominal 3.12 (2.90 µg ai/L) = 100 µg formulation/L nominal 7.58 (5.80 µg ai/L) = 200 µg formulation/L nominal							

B. NON-LETHAL TOXICITY ENDPOINTS:

Sub-lethal effects were restricted to the two highest treatment levels and included surfacing, lying on the bottom of the test chamber, and loss of equilibrium.

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

Table 4: Sub-lethal Effect of Permethrin TEP on *Lepomis macrochirus*.

Mean-Measured and (Nominal) Permethrin Concentrations µg ai/L	Observation Period- combined replicates		
	24 Hours	48 Hours	96 Hours
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
Solvent Control	A.N.	A.N.	A.N.
0.434 (0.377 µg ai/L – 13 µg formulation/L)	A.N.	A.N.	A.N.
0.956 (0.725 µg ai/L -25 µg formulation/L)	A.N.	A.N.	A.N.
1.48 (1.45 µg ai/L- 50 µg formulation/L)	A.N.	A.N.	A.N.
3.12 (2.90 µg ai/L- 100 µg formulation/L)	A.N.	26%- surfacing	100%-surfacing
7.58 (5.80 µg ai/L- 200 µg formulation/L)	10%- surfacing	50%- loss of equilibrium 13%- surfacing 38%- lying on bottom	-- ¹
EC ₅₀	Not Reported		
Positive control, if used % sublethal effect: EC ₅₀ :	N/A		

¹ Complete mortality

C. REPORTED STATISTICS:

The 96-hour LC₅₀ value and 95% C.I. were estimated using the probit analysis from the computer program of C.E. Stephan. Toxicity values were based on the mean-measured permethrin concentrations.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer estimated the 96-hour LC₅₀ and associated 95% C.I. using the probit analysis via CETIS statistical software version 1.8.7.12 with database backend settings implemented by EFED on 3/25/14. Three separate analyses were conducted: based on TWA permethrin concentration, based on the TWA PBO concentrations, and based on the TWA permethrin + PBO concentrations.

Permethrin + PBO:

LC₅₀: 11.8 µg ai/L 95% C.I.: 9.85-14.0 µg ai/L
Probit Slope: 9.26 95% C.I.: 5.60-12.9

Permethrin:

LC₅₀: 2.12 µg ai/L 95% C.I.: 1.78-2.53 µg ai/L
Probit Slope: 9.35 95% C.I.: 5.70-13.0

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

PBO:

LC₅₀: 9.67 µg ai/L 95% C.I.: 8.09-11.6 µg ai/L
Probit Slope: 9.19 95% C.I.: 5.55-12.8

E. STUDY DEFICIENCIES: Though some departures from recommended test procedures are noted in Materials and Methods section above they were not felt to have affected the reported results.

F. REVIEWER'S COMMENTS:

The reviewer's results based on the permethrin concentrations were comparable to those reported by the study authors. However, the reviewer also estimated toxicity values based on the TWA PBO and TWA permethrin + PBO concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The reviewer estimated the TWA concentrations for each separate active ingredient using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C TWA is the time-weighted average concentration,

C j is the concentration measured at time interval j (j = 0, 1, 2,...n)

t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j

(e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours)

The measured concentrations for permethrin were much more variable for permethrin (CVs of 10.6-37.8%) than for PBO (CVs of 1.4-20.2%). However, the reviewer estimated TWA for PBO as well. Only the nominal 1.86 and 7.15 µg PBO/L had high variation (CVs of 15.2 and 20.2%, respectively), however, the TWA concentrations were comparable to the mean-measured concentrations. Therefore, the reviewer feels that the TWA concentrations for PBO are appropriate to use.

Results from the periodic screening analysis of the dilution water indicated the presence of the following analytes: calcium (33.5 mg/L), chloride (3.7 mg/L), magnesium (12.8 mg/L), potassium (6.60 mg/L), and sodium (17.8 mg/L).

The in-life portion of the definitive toxicity test was conducted from September 12 to 16, 2014.

G. CONCLUSIONS:

This study is scientifically sound for evaluation of toxicity of the Permethrin/Piperonyl butoxide formulation ratio tested and may be used to characterize potential toxicity of this mixture to fresh, warm water fish species as represented by the Bluegill sunfish.

Data Evaluation Record on the Acute Toxicity of Permethrin TEP (High PBO:Permethrin Ratio) to Fish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 49540301

III. REFERENCES:

Stephan CE. 1982. US EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal Communication.

Peltier WH Weber CI. 1985. "Methods for Measuring the Acute Toxicity of Effluences to Freshwater and Marine Organisms." United States Environmental Protection Agency. EPA/600/4-85/013. Pp. 216.

Finney DJ. 1971. *Statistical Methods in Biological Assay*. Second Edition. Griffin Press, London.

CETIS Summary Report

Report Date: 16 Apr-15 13:31 (p 1 of 1)

Test Code: 49540301 PERPBO | 07-8534-2047

OPPTS 850.1075 Acute Fish

Wildlife International

Batch ID:	11-9225-4261	Test Type:	Mortality (96-h)	Analyst:	
Start Date:	14 Sep-14	Protocol:	OPPTS 850.1075 Acute Fish	Diluent:	Well Water
Ending Date:		Species:	Lepomis macrochirus	Brine:	Not Applicable
Duration:	NA	Source:	Osage Catfisheries, Osage Beach, MI	Age:	0.3g
Sample ID:	05-4142-1155	Code:	49540301 PERPBO	Client:	CDM Smith
Sample Date:	14 Sep-14	Material:	Permethrin + PBO	Project:	Insecticide
Receive Date:		Source:	Consumer Specialty Products (CONTASKF		
Sample Age:	NA	Station:			

Batch Note: PC Code 109701 MRID 49540301 TWA Permethrin + PBO

Sample Note: PC Code 109701 MRID 49540301 TWA Permethrin + PBO

Point Estimate Summary

Analysis ID	Endpoint	Level	µg al/L	95% LCL	95% UCL	TU	Method
21-0559-7743	96h Mortality Rate	LC5	7.81	5.58	9.4		Linear Regression (MLE)
		LC10	8.55	6.39	10.2		
		LC15	9.09	6.99	10.7		
		LC20	9.54	7.5	11.2		
		LC25	9.94	7.94	11.7		
		LC40	11	9.12	13.1		
		LC50	11.8	9.85	14		
09-8343-9170	96h Mortality Rate	LC50	11.8	10.7	13		Spearman-Kärber

96h Mortality Rate Summary

C-µg al/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	2	0	0	0	0	0	0	0		
0	Negative Control	2	0	0	0	0	0	0	0		
2.33		2	0	0	0	0	0	0	0		
4.4		2	0	0	0	0	0	0	0		
7.81		2	0.05	0	0.685	0	0.1	0.05	0.0707	141.0%	
17.7		2	0.95	0.315	1	0.9	1	0.05	0.0707	7.44%	
34.3		2	1	1	1	1	1	0	0	0.0%	

96h Mortality Rate Detail

C-µg al/L	Control Type	Rep 1	Rep 2
0	Solvent Blank	0	0
0	Negative Control	0	0
2.33		0	0
4.4		0	0
7.81		0.1	0
17.7		1	0.9
34.3		1	1

CETIS Summary Report

Report Date: 16 Apr-15 13:09 (p 1 of 1)

Test Code: 109701 49540301 | 19-6859-0087

OPPTS 850.1075 Acute Fish

Wildlife International

Batch ID:	02-6499-0149	Test Type:	Mortality (96-h)	Analyst:	
Start Date:	12 Sep-14	Protocol:	OPPTS 850.1075 Acute Fish	Diluent:	Well Water
Ending Date:		Species:	Lepomis macrochirus	Brine:	Not Applicable
Duration:	NA	Source:	Osage Catfisheries, Osage Beach, MI	Age:	0.3g
Sample ID:	01-6445-5981	Code:	109701 49540301	Client:	CDM Smith
Sample Date:	12 Sep-14	Material:	Permethrin	Project:	Insecticide
Receive Date:		Source:	Consumer Specialty Products (CONTASKF		
Sample Age:	NA	Station:			

Batch Note: PC Code 109701 MRID 49540301 Permethrin TWA

Sample Note: PC Code 109701 MRID 49540301 Permethrin TWA

Point Estimate Summary

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
16-4161-5642	96h Mortality Rate	LC5	1.41	1.02	1.69		Linear Regression (MLE)
		LC10	1.55	1.17	1.83		
		LC15	1.64	1.27	1.93		
		LC20	1.72	1.36	2.02		
		LC25	1.79	1.44	2.11		
		LC40	1.99	1.65	2.35		
		LC50	2.12	1.78	2.53		
11-2105-2237	96h Mortality Rate	LC50	2.14	1.93	2.37		Spearman-Kärber

96h Mortality Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	2	0	0	0	0	0	0	0		
0	Negative Control	2	0	0	0	0	0	0	0		
0.471		2	0	0	0	0	0	0	0		
0.916		2	0	0	0	0	0	0	0		
1.41		2	0.05	0	0.685	0	0.1	0.05	0.0707	141.0%	
3.18		2	0.95	0.315	1	0.9	1	0.05	0.0707	7.44%	
7.58		2	1	1	1	1	1	0	0	0.0%	

96h Mortality Rate Detail

C-µg ai/L	Control Type	Rep 1	Rep 2
0	Solvent Blank	0	0
0	Negative Control	0	0
0.471		0	0
0.916		0	0
1.41		0.1	0
3.18		1	0.9
7.58		1	1

CETIS Summary Report

Report Date: 16 Apr-15 13:22 (p 1 of 1)

Test Code: 49540301 PBO | 05-1975-8379

OPPTS 850.1075 Acute Fish

Wildlife International

Batch ID: 19-3535-3307	Test Type: Mortality (96-h)	Analyst:
Start Date: 13 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

Sample ID: 00-7591-8397	Code: 49540301 PBO	Client: CDM Smith
Sample Date: 13 Sep-14	Material: Piperonyl butoxide	Project: Insecticide
Receive Date:	Source: Consumer Specialty Products (CONTASKF	
Sample Age: NA	Station:	

Batch Note: PC Code 109701 MRID 49540301 TWA PBO

Sample Note: PC Code 109701 MRID 49540301 PBO TWA

Point Estimate Summary

Analysis ID	Endpoint	Level	µg ai/L	95% LCL	95% UCL	TU	Method
04-1864-1580	96h Mortality Rate	LC5	6.4	4.56	7.72		Linear Regression (MLE)
		LC10	7.01	5.23	8.34		
		LC15	7.46	5.72	8.82		
		LC20	7.83	6.14	9.23		
		LC25	8.16	6.51	9.62		
		LC40	9.07	7.48	10.7		
		LC50	9.67	8.09	11.6		
07-9256-9098	96h Mortality Rate	LC50	9.67	8.76	10.7		Spearman-Kärber

96h Mortality Rate Summary

C-µg ai/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Solvent Blank	2	0	0	0	0	0	0	0		
0	Negative Control	2	0	0	0	0	0	0	0		
1.86		2	0	0	0	0	0	0	0		
3.48		2	0	0	0	0	0	0	0		
6.4		2	0.05	0	0.685	0	0.1	0.05	0.0707	141.0%	
14.6		2	0.95	0.315	1	0.9	1	0.05	0.0707	7.44%	
26.8		2	1	1	1	1	1	0	0	0.0%	

96h Mortality Rate Detail

C-µg ai/L	Control Type	Rep 1	Rep 2
0	Solvent Blank	0	0
0	Negative Control	0	0
1.86		0	0
3.48		0	0
6.4		0.1	0
14.6		1	0.9
26.8		1	1

CETIS Analytical Report

Report Date: 16 Apr-15 13:30 (p 1 of 2)

Test Code: 49540301 PERPBO | 07-8534-2047

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 21-0559-7743	Endpoint: 96h Mortality Rate	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-15 13:29	Analysis: Linear Regression (MLE)	Official Results: Yes
Batch ID: 11-9225-4261	Test Type: Mortality (96-h)	Analyst:
Start Date: 14 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	No	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
5	-7.94	21.2	20.9	1.07	0.108	0.979	0.00386	4.53	1.0000	Non-Significant Lack of Fit

Point Estimates

Level	µg ai/L	95% LCL	95% UCL
LC5	7.81	5.58	9.4
LC10	8.55	6.39	10.2
LC15	9.09	6.99	10.7
LC20	9.54	7.5	11.2
LC25	9.94	7.94	11.7
LC40	11	9.12	13.1
LC50	11.8	9.85	14

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Slope	9.26	1.87	5.6	12.9	4.95	0.0006	Significant Parameter
Intercept	-9.92	2.03	-13.9	-5.94	-4.89	0.0006	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	109.7893	109.7893	1	520	<0.0001	Significant
Lack of Fit	0.005422	0.001356	4	0.00386	1.0000	Non-Significant
Pure Error	2.105263	0.350877	6			
Residual	2.110685	0.211069	10			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	2.11	18.3	0.9954	Non-Significant Heterogeneity
	Likelihood Ratio GOF	2.88	18.3	0.9841	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	65500	4.39	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.789	0.861	0.0070	Non-normal Distribution
	Anderson-Darling A2 Normality	1.59	2.49	<0.0001	Non-normal Distribution

96h Mortality Rate Summary

		Calculated Variate(A/B)									
C-µg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	2	0	0	0	0	0			0	20
2.33		2	0	0	0	0	0			0	20
4.4		2	0	0	0	0	0			0	20
7.81		2	0.05	0	0.1	0.05	0.0707	141.0%		1	20
17.7		2	0.95	0.9	1	0.05	0.0707	7.44%		19	20
34.3		2	1	1	1	0	0	0.0%		20	20

CETIS Analytical Report

Report Date: 16 Apr-15 13:08 (p 1 of 2)

Test Code: 109701 49540301 | 19-6859-0087

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 16-4161-5642	Endpoint: 96h Mortality Rate	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-15 13:07	Analysis: Linear Regression (MLE)	Official Results: Yes
Batch ID: 02-6499-0149	Test Type: Mortality (96-h)	Analyst:
Start Date: 12 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	No	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
5	-7.95	21.2	20.9	0.326	0.107	0.979	0.0216	4.53	0.9990	Non-Significant Lack of Fit

Point Estimates

Level	µg al/L	95% LCL	95% UCL
LC5	1.41	1.02	1.69
LC10	1.55	1.17	1.83
LC15	1.64	1.27	1.93
LC20	1.72	1.36	2.02
LC25	1.79	1.44	2.11
LC40	1.99	1.65	2.35
LC50	2.12	1.78	2.53

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Slope	9.35	1.86	5.7	13	5.03	0.0005	Significant Parameter
Intercept	-3.05	0.687	-4.39	-1.7	-4.44	0.0013	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	109.7644	109.7644	1	514	<0.0001	Significant
Lack of Fit	0.030377	0.007594	4	0.0216	0.9988	Non-Significant
Pure Error	2.105263	0.350877	6			
Residual	2.135641	0.213564	10			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	2.14	18.3	0.9952	Non-Significant Heterogeneity
	Likelihood Ratio GOF	2.89	18.3	0.9839	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	65500	4.39	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.805	0.861	0.0106	Non-normal Distribution
	Anderson-Darling A2 Normality	1.46	2.49	0.0003	Non-normal Distribution

96h Mortality Rate Summary

		Calculated Variate(A/B)									
C-µg al/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	2	0	0	0	0	0			0	20
0.471		2	0	0	0	0	0			0	20
0.916		2	0	0	0	0	0			0	20
1.41		2	0.05	0	0.1	0.05	0.0707	141.0%		1	20
3.18		2	0.95	0.9	1	0.05	0.0707	7.44%		19	20
7.58		2	1	1	1	0	0	0.0%		20	20

CETIS Analytical Report

Report Date: 16 Apr-15 13:21 (p 1 of 2)

Test Code: 49540301 PBO | 05-1975-8379

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 04-1864-1580	Endpoint: 96h Mortality Rate	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-15 13:20	Analysis: Linear Regression (MLE)	Official Results: Yes
Batch ID: 19-3535-3307	Test Type: Mortality (96-h)	Analyst:
Start Date: 13 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Hat Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	1E-07	No	No	No	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
5	-7.94	21.2	20.9	0.985	0.109	0.979	0.00392	4.53	1.0000	Non-Significant Lack of Fit

Point Estimates

Level	µg al/L	95% LCL	95% UCL
LC5	6.4	4.56	7.72
LC10	7.01	5.23	8.34
LC15	7.46	5.72	8.82
LC20	7.83	6.14	9.23
LC25	8.16	6.51	9.62
LC40	9.07	7.48	10.7
LC50	9.67	8.09	11.6

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Slope	9.19	1.86	5.55	12.8	4.95	0.0006	Significant Parameter
Intercept	-9.06	1.86	-12.7	-5.41	-4.87	0.0007	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	109.7892	109.7892	1	520	<0.0001	Significant
Lack of Fit	0.005497	0.001374	4	0.00392	1.0000	Non-Significant
Pure Error	2.105263	0.350877	6			
Residual	2.11076	0.211076	10			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	2.11	18.3	0.9954	Non-Significant Heterogeneity
	Likelihood Ratio GOF	2.88	18.3	0.9841	Non-Significant Heterogeneity
Variances	Mod Levene Equality of Variance	65500	4.39	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.789	0.861	0.0071	Non-normal Distribution
	Anderson-Darling A2 Normality	1.58	2.49	<0.0001	Non-normal Distribution

96h Mortality Rate Summary

		Calculated Variate(A/B)									
C-µg al/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	2	0	0	0	0	0			0	20
1.86		2	0	0	0	0	0			0	20
3.48		2	0	0	0	0	0			0	20
6.4		2	0.05	0	0.1	0.05	0.0707	141.0%		1	20
14.6		2	0.95	0.9	1	0.05	0.0707	7.44%		19	20
26.8		2	1	1	1	0	0	0.0%		20	20

CETIS Analytical Report

Report Date: 16 Apr-15 13:21 (p 1 of 1)

Test Code: 49540301 PBO | 05-1975-8379

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 07-9256-9098	Endpoint: 96h Mortality Rate	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-15 13:20	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Batch ID: 19-3535-3307	Test Type: Mortality (96-h)	Analyst:
Start Date: 13 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

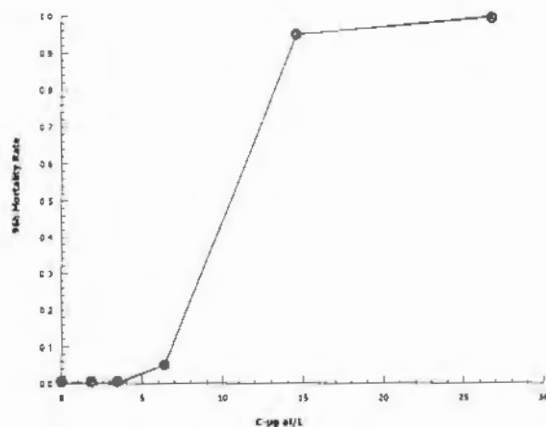
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	0.985	0.0214	9.67	8.76	10.7

96h Mortality Rate Summary

		Calculated Variate(A/B)								
C-µg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A B
0	Negative Control	2	0	0	0	0	0			0 20
1.86		2	0	0	0	0	0			0 20
3.48		2	0	0	0	0	0			0 20
6.4		2	0.05	0	0.1	0.05	0.0707	141.0%		1 20
14.6		2	0.95	0.9	1	0.05	0.0707	7.44%		19 20
26.8		2	1	1	1	0	0	0.0%		20 20

Graphics



CETIS Analytical Report

Report Date: 16 Apr-15 13:30 (p 1 of 1)

Test Code: 49540301 PERPBO | 07-8534-2047

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 09-8343-9170	Endpoint: 96h Mortality Rate	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-15 13:29	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Batch ID: 11-9225-4261	Test Type: Mortality (96-h)	Analyst:
Start Date: 14 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

Spearman-Kärber Estimates

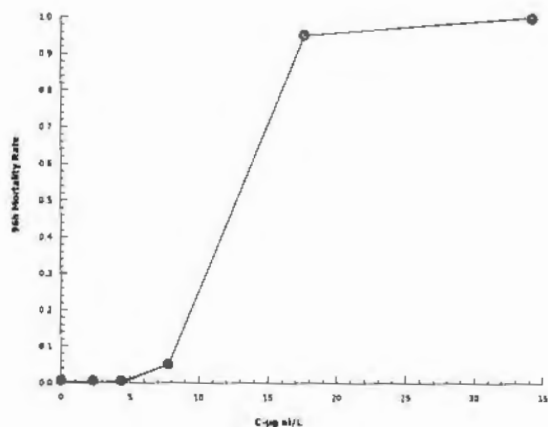
Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	1.07	0.0215	11.8	10.7	13

96h Mortality Rate Summary

Calculated Variate(A/B)

C-µg al/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	2	0	0	0	0	0			0	20
2.33		2	0	0	0	0	0			0	20
4.4		2	0	0	0	0	0			0	20
7.81		2	0.05	0	0.1	0.05	0.0707	141.0%		1	20
17.7		2	0.95	0.9	1	0.05	0.0707	7.44%		19	20
34.3		2	1	1	1	0	0	0.0%		20	20

Graphics



CETIS Analytical Report

Report Date: 16 Apr-15 13:08 (p 1 of 1)

Test Code: 109701 49540301 | 19-6859-0087

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 11-2105-2237	Endpoint: 96h Mortality Rate	CETIS Version: CETISv1.8.7
Analyzed: 16 Apr-15 13:07	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes
Batch ID: 02-6499-0149	Test Type: Mortality (96-h)	Analyst:
Start Date: 12 Sep-14	Protocol: OPPTS 850.1075 Acute Fish	Diluent: Well Water
Ending Date:	Species: Lepomis macrochirus	Brine: Not Applicable
Duration: NA	Source: Osage Catfisheries, Osage Beach, MI	Age: 0.3g

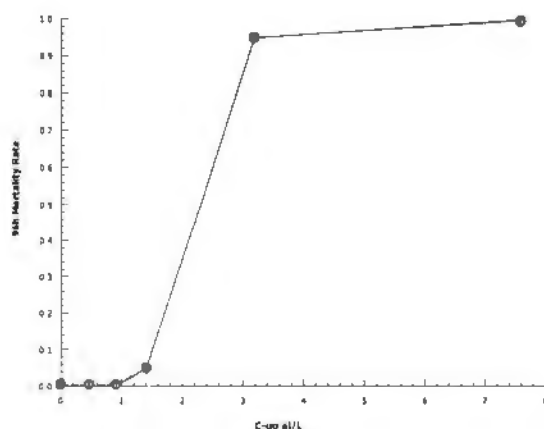
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	LC50	95% LCL	95% UCL
Control Threshold	0	0.00%	0.331	0.0221	2.14	1.93	2.37

96h Mortality Rate Summary

		Calculated Variate(A/B)									
C-µg ai/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Negative Control	2	0	0	0	0	0			0	20
0.471		2	0	0	0	0	0			0	20
0.916		2	0	0	0	0	0			0	20
1.41		2	0.05	0	0.1	0.05	0.0707	141.0%		1	20
3.18		2	0.95	0.9	1	0.05	0.0707	7.44%		19	20
7.58		2	1	1	1	0	0	0.0%		20	20

Graphics



CETIS Analytical Report

Report Date: 16 Apr-15 13:21 (p 2 of 2)

Test Code: 49540301 PBO | 05-1975-8379

OPPTS 850.1075 Acute Fish

Wildlife International

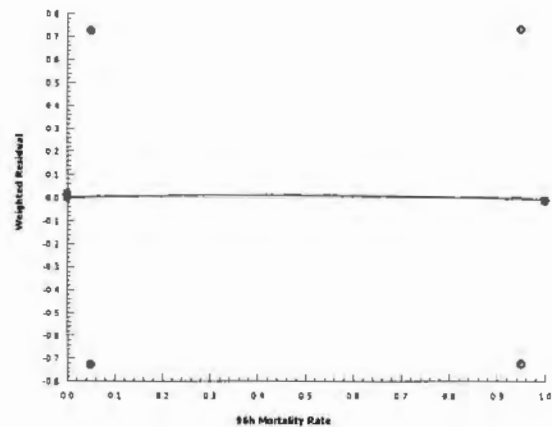
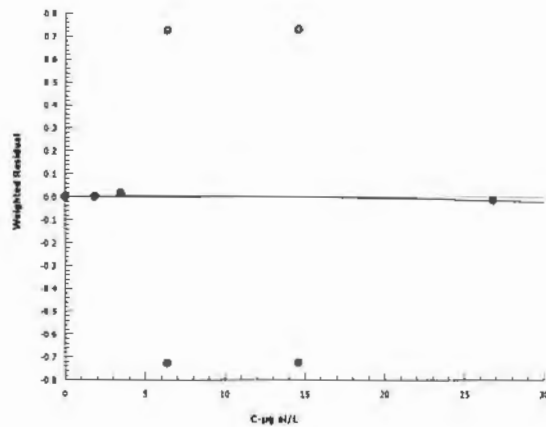
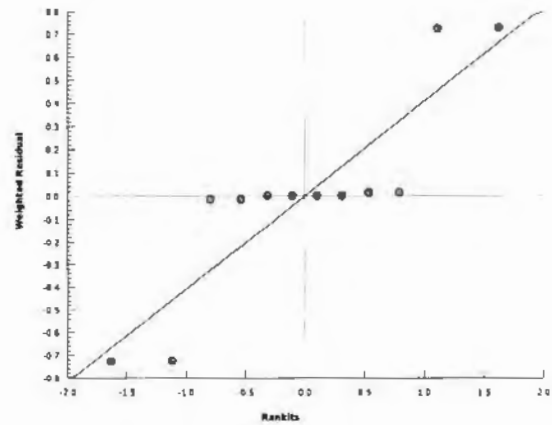
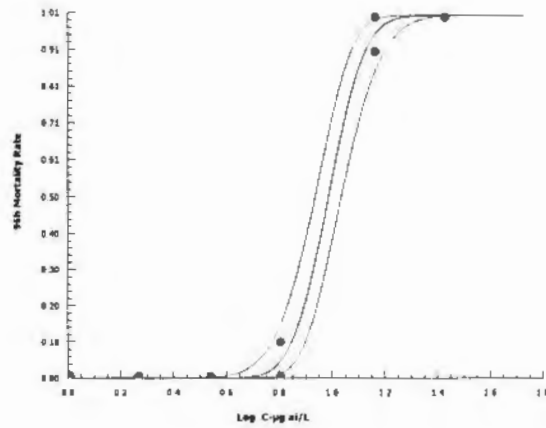
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Analyzed: 16 Apr-15 13:20

Endpoint: 96h Mortality Rate
Analysis: Linear Regression (MLE)

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics

Log-Normal [NED=A+B*log(X)]



CETIS Analytical Report

Report Date: 16 Apr-15 13:08 (p 2 of 2)

Test Code: 109701 49540301 | 19-6859-0087

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 16-4161-5642

Endpoint: 96h Mortality Rate

CETIS Version: CETISv1.8.7

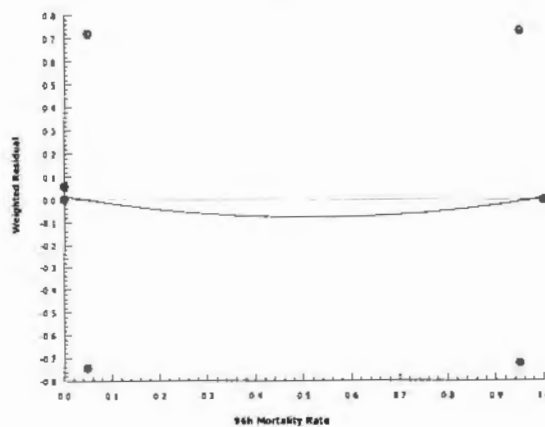
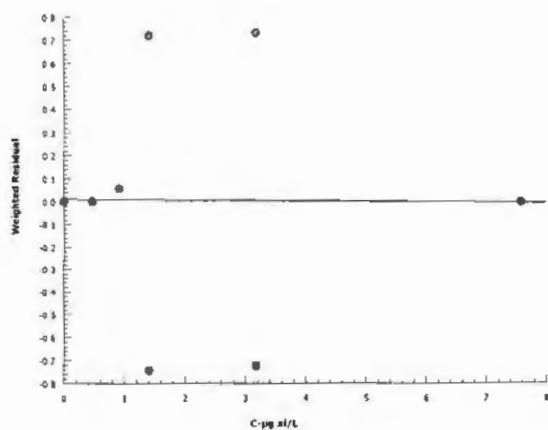
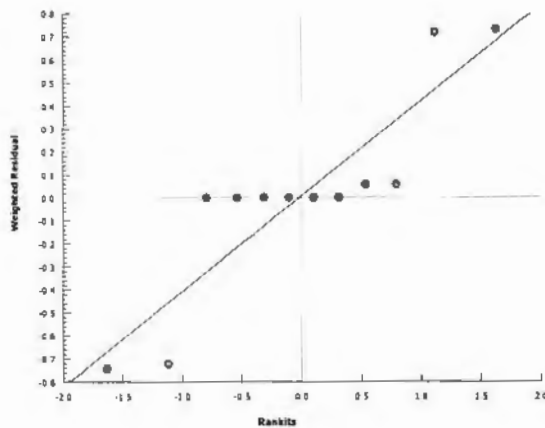
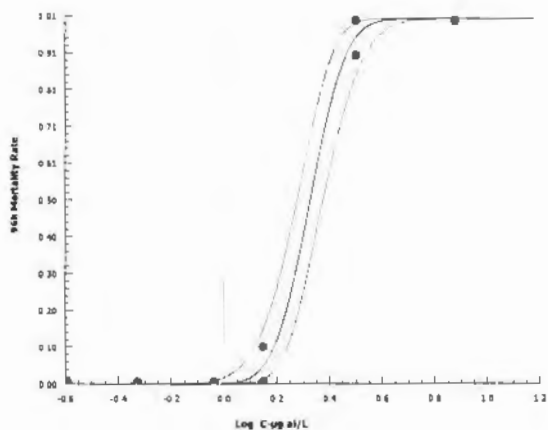
Analyzed: 16 Apr-15 13:07

Analysis: Linear Regression (MLE)

Official Results: Yes

Graphics

Log-Normal [NED=A+B*log(X)]



CETIS Analytical Report

Report Date: 16 Apr-15 13:30 (p 2 of 2)

Test Code: 49540301 PERPBO | 07-8534-2047

OPPTS 850.1075 Acute Fish

Wildlife International

Analysis ID: 21-0559-7743

Endpoint: 96h Mortality Rate

CETIS Version: CETISv1.8.7

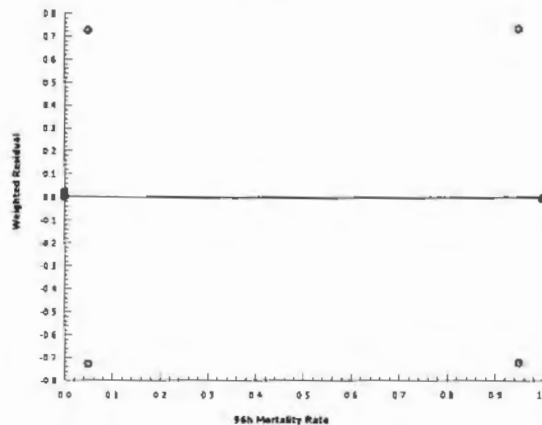
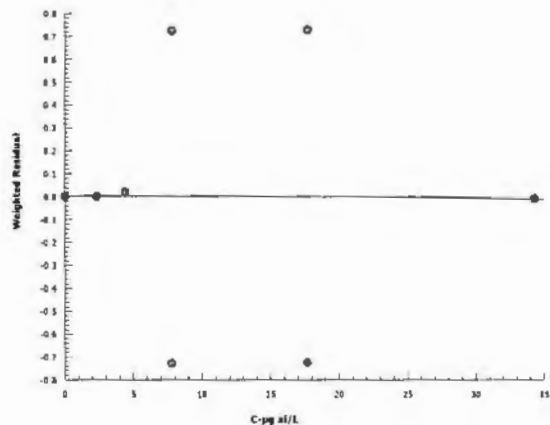
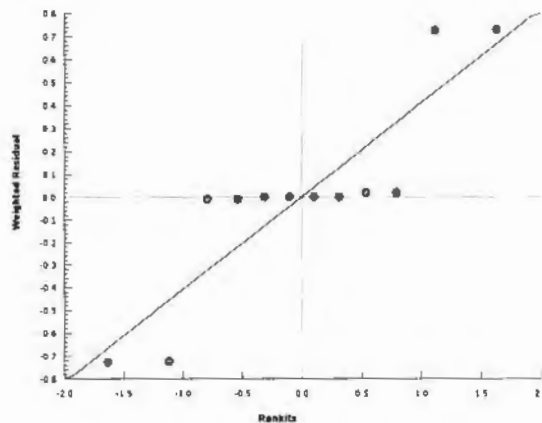
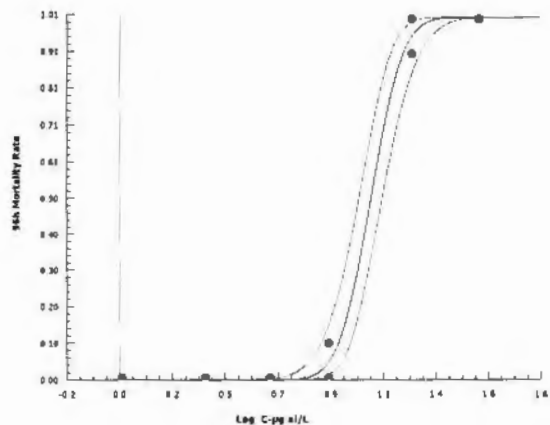
Analyzed: 16 Apr-15 13:29

Analysis: Linear Regression (MLE)

Official Results: Yes

Graphics

Log-Normal [NED=A+B*log(X)]



- 23 -

Table 1

Stock Solution Analysis for Permethrin

Nominal Formulation Concentration ($\mu\text{g/mL}$)	Nominal Permethrin Concentration ($\mu\text{g a.i./mL}$)	Sample Number (701A-115-)	Measured Permethrin Concentration ($\mu\text{g a.i./mL}$) ¹	Percent of Nominal
130	3.77	ST-1	4.20	111
		ST-6	4.26	113
		ST-11	4.22	112
250	7.25	ST-2	7.96	110
		ST-7	9.11	126
		ST-12	8.64	119
500	14.5	ST-3	16.1	111
		ST-8	16.0	110
		ST-13	17.1	118
1000	29.0	ST-4	32.5	112
		ST-9	29.4	102
		ST-14	35.1	121
2000	58.0	ST-5	65.8	113
		ST-10	57.3	98.8
		ST-15	69.1	119
500	14.5	² PT-MAS-1-ST	16.3	113

¹Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

²Matrix fortification sample analyzed concurrently with stock solutions.

- 24 -

Table 2

Stock Solution Analysis for PBO

Nominal Formulation Concentration (µg/mL)	Nominal PBO Concentration (µg a.i./mL)	Sample Number (701A-115-)	Measured PBO Concentration (µg a.i./mL) ^{1,3}	Percent of Nominal
130	18.6	ST-1	18.5	99.6
		ST-6	18.3	98.6
		ST-11	18.0	97.1
250	35.7	ST-2	35.6	99.8
		ST-7	39.0	109
		ST-12	37.0	104
500	71.5	ST-3	71.9	101
		ST-8	69.6	97.4
		ST-13	74.0	104
1000	143	ST-4	145	101
		ST-9	146	102
		ST-14	153	107
2000	286	ST-5	289	101
		ST-10	280	97.8
		ST-15	300	105
500	71.5	² PT-MAS-1-ST	72.5	102

¹ Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

² Matrix fortification sample analyzed concurrently with stock solutions.

³ The ratio of mean measured concentrations of permethrin to PBO ranged from 1: 3.5 to 1: 4.7.

- 25 -

Table 3

Measured Concentrations of Permethrin in Pretest Diluter Verification Samples

Nominal Formulation Concentration (µg/L)	Nominal Permethrin Concentration (µg a.i./L)	Sample Number (701A-115-)	Sampling Time (Day)	Measured Permethrin Concentration (µg a.i./L) ^{1,3}	Percent of Nominal ²
Negative Control	Negative Control	PT-15	-7	< LOQ	--
		PT-29	-2	< LOQ	--
Solvent Control	Solvent Control	PT-16	-7	< LOQ	--
		PT-30	-2	< LOQ	--
13	0.377	PT-17	-7	0.339	89.8
		PT-31	-2	0.406	108
25	0.725	PT-18	-7	1.02	141
		PT-32	-2	0.831	115
50	1.45	PT-19	-7	1.69	116
		PT-33	-2	1.61	111
100	2.90	PT-20	-7	3.73	129
		PT-34	-2	4.24	146
200	5.80	PT-21	-7	9.64	166
		PT-35	-2	8.35	144

¹ The limit of quantitation (LOQ) was 0.240 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (4.00 µg a.i./L) and the dilution factor of the matrix blank samples (0.0600).

² Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

³ The ratio of mean measured concentrations of permethrin to PBO ranged from 1: 3.5 to 1: 4.7.



- 26 -

Table 4

Measured Concentrations of PBO in Pretest Diluter Verification Samples

Nominal Formulation Concentration (µg/L)	Nominal PBO Concentration (µg a.i./L)	Sample Number (701A-115-)	Sampling Time (Day)	Measured PBO Concentration (µg a.i./L) ¹	Percent of Nominal ²
Negative Control	Negative Control	PT-15	-7	< LOQ	--
		PT-29	-2	< LOQ	--
Solvent Control	Solvent Control	PT-16	-7	< LOQ	--
		PT-30	-2	< LOQ	--
13	1.86	PT-17	-7	2.04	110
		PT-31	-2	2.28	123
25	3.57	PT-18	-7	4.05	113
		PT-32	-2	4.06	114
50	7.15	PT-19	-7	7.51	105
		PT-33	-2	8.25	115
100	14.3	PT-20	-7	14.4	101
		PT-34	-2	16.9	118
200	28.6	PT-21	-7	27.5	96.4
		PT-35	-2	29.9	105

¹ The limit of quantitation (LOQ) was 1.20 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (20.0 µg a.i./L) and the dilution factor of the matrix blank samples (0.0600).

² Results were generated using Excel 2010 in full precision mode. Manual calculations may differ slightly.

- 27 -

Table 5

Measured Concentrations of Permethrin in Test Solution Samples

Nominal Formulation Concentration (µg/L)	Nominal Permethrin Concentration (µg a.i./L)	Sample Number (701A-115-)	Sampling Time (Hours)	Measured Permethrin Concentration (µg a.i./L) ¹	Percent of Nominal ²	Mean Measured Permethrin Concentration (µg a.i./L)	Mean Permethrin Measured Percent of Nominal
Negative Control	Negative Control	1	0	< LOQ	--	< LOQ	--
		10	48	< LOQ	--		
		17	96	< LOQ	--		
Solvent Control	Solvent Control	2	0	< LOQ	--	< LOQ	--
		11	48	< LOQ	--		
		18	96	< LOQ	--		
13	0.377	3	0	0.336	89.2	0.434 ± 0.129 CV = 29.7%	115
		12	48	0.580	154		
		19	96	0.386	102		
25	0.725	4	0	0.704	97.2	0.956 ± 0.361 CV = 37.8%	132
		13	48	0.794	110		
		20	96	1.37	189		
50	1.45	5	0	1.51	104	1.48 ± 0.266 CV = 18.0%	102
		14	48	1.20	82.5		
		21	96	1.73	119		
100	2.90	6	0	3.39	117	3.12 ± 0.465 CV = 14.9%	108
		15	48	3.38	116		
		22	96	2.58	88.9		
200	5.80	7	0	7.01	121	7.58 ± 0.806 CV = 10.6%	131
		16	48	8.15	140		
		23	96	17.9**	309*		

¹ The limit of quantitation (LOQ) was 0.240 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (4.00 µg a.i./L) and the dilution factor of the matrix blank samples (0.0600).

² Results were generated using Excel 2010 in the full precision mode. Manual calculations may differ slightly.

* Not included in mean measured calculation. Analysis for PBO at 96 hours indicates solutions were being delivered at the approximate nominal concentration. This value is a statistical outlier.

** Extrapolated value.

- 28 -

Table 6

Measured Concentrations of PBO in Test Solution Samples

Nominal Formulation Concentration (µg/L)	Nominal PBO Concentration (µg a.i./L)	Sample Number (701A-115-)	Sampling Time (Hours)	Measured PBO Concentration (µg a.i./L) ¹	Percent of Nominal ²	Mean Measured PBO Concentration (µg a.i./L)	Mean Measured Percent of Nominal
Negative Control	Negative Control	1	0	< LOQ	--	< LOQ	--
		10	48	< LOQ	--		
		17	96	< LOQ	--		
Solvent Control	Solvent Control	2	0	< LOQ	--	< LOQ	--
		11	48	< LOQ	--		
		18	96	< LOQ	--		
13	1.86	3	0	2.07	111	1.95 ± 0.295 CV = 15.2%	105
		12	48	1.61	86.7		
		19	96	2.16	116		
25	3.57	4	0	3.45	96.5	3.50 ± 0.099 CV = 2.82%	98.0
		13	48	3.43	95.9		
		20	96	3.61	101		
50	7.15	5	0	8.04	113	6.76 ± 1.37 CV = 20.2	94.5
		14	48	5.32	74.5		
		21	96	6.92	96.9		
100	14.3	6	0	14.8	103	14.6 ± 0.200 CV = 1.37%	102
		15	48	14.4	101		
		22	96	14.6	102		
200	28.6	7	0	27.1	95.0	26.8 ± 0.945 CV = 3.53%	93.7
		16	48	25.7	90.0		
		23	96	27.5	96.1		

¹ The limit of quantitation (LOQ) was 1.20 µg a.i./L, calculated as the product of the concentration of the lowest calibration standard (20.0 µg a.i./L) and the dilution factor of the matrix blank samples (0.0600).

² Results were generated using Excel 2010 in the full precision mode. Manual calculations may differ slightly.



- 29 -

Table 7

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Mean Measured Concentration (µg Permethrin a.i./L)	Replicate	~ 4.5 Hour			24 Hours			48 Hours		
		Temperature ¹ (°C)	DO ² (mg/L)	pH	Temperature ¹ (°C)	DO ² (mg/L)	pH	Temperature ¹ (°C)	DO ² (mg/L)	pH
Negative Control	A	21.9	8.7	8.1	21.8	--	--	21.9	8.7	8.1
	B	21.9	--	--	21.8	8.6	8.1	21.9	--	--
Solvent Control	A	21.9	8.6	8.1	21.8	--	--	21.8	8.1	8.1
	B	22.0	--	--	21.8	8.0	8.1	21.9	--	--
0.434	A	21.9	8.2	8.0	21.8	--	--	21.8	7.8	8.1
	B	21.9	--	--	21.7	7.7	8.1	21.9	--	--
0.956	A	21.9	8.5	8.1	21.8	--	--	21.8	8.3	8.1
	B	21.9	--	--	21.7	8.1	8.2	21.8	--	--
1.48	A	21.9	8.5	8.1	21.8	--	--	21.8	8.3	8.1
	B	21.8	--	--	21.7	7.9	8.1	21.7	--	--
3.12	A	21.9	7.7	8.0	21.8	--	--	21.8	7.9	8.1
	B	21.9	--	--	21.8	7.7	8.1	21.9	--	--
7.58	A	21.9	8.1	8.0	21.8	--	--	21.8	8.1	8.1
	B	21.9	--	--	21.7	7.7	8.1	21.8	--	--

¹ Manual temperature measurements. Temperature monitored continuously during the test ranged from 21.88 to 22.01°C, measured to the nearest 0.01°C.

² A dissolved oxygen concentration of 8.7 mg/L represents 100% saturation at 22°C in freshwater.

-- No measurement scheduled.

- 30 -

Table 7 (Continued)

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Mean Measured Concentration (µg Permethrin a.i./L)	Replicate	72 Hours			96 Hours		
		Temperature ¹ (°C)	DO ² (mg/L)	pH	Temperature ¹ (°C)	DO ² (mg/L)	pH
Negative Control	A	--	--	--	22.0	8.7	8.0
	B	--	8.7	8.1	22.0	--	--
Solvent Control	A	--	--	--	22.0	7.5	8.0
	B	--	7.8	8.0	22.0	--	--
0.434	A	--	--	--	-- ⁴	7.1	8.0
	B	--	7.7	8.0	-- ⁴	--	--
0.956	A	--	--	--	-- ⁴	7.4	8.0
	B	--	8.2	8.1	-- ⁴	--	--
1.48	A	--	--	--	22.0	7.5	8.0
	B	--	7.9	8.0	22.0	--	--
3.12	A	--	--	--	22.0	7.4	8.0
	B	--	7.9	8.0	22.0	--	--
7.58	A	21.8 ³	7.9 ³	8.0 ¹	--	--	--
	B	21.8 ³	7.8 ³	8.0 ³	--	--	--

¹ Manual temperature measurements. Temperature monitored continuously during the test ranged from 21.88 to 22.01°C, measured to the nearest 0.01°C.

² A dissolved oxygen concentration of 8.7 mg/L represents 100% saturation at 22°C in freshwater.

³ Final measurements were taken at 72 hours and then discontinued due to 100% mortality in the test chamber.

⁴ Value not reported due to a data recording error.

-- No measurement scheduled.

- 31 -

Table 8

Specific Conductance, Hardness and Alkalinity
in the Dilution Water at Test Initiation

Parameter	Day 0
Specific Conductance ($\mu\text{S}/\text{cm}$)	334
Hardness (mg/L as CaCO_3)	132
Alkalinity (mg/L as CaCO_3)	182

- 32 -

Table 9

Cumulative Mortality and Observations

Mean Measured Concentration (μ g Permethrin a.i./L)	Rep.	No. Exposed	~ 4.5 Hours		24 Hours		48 Hours	
			No. Dead ¹	Observations ²	No. Dead ¹	Observations ²	No. Dead ¹	Observations ²
Negative Control	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
Solvent Control	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
0.434	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
0.956	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
1.48	A	10	0	10 AN	0	10 AN	0	10 AN
	B	10	0	10 AN	0	10 AN	0	10 AN
3.12	A	10	0	10 AN	0	10 AN	1	2 A; 7 AN
	B	10	0	10 AN	0	10 AN	0	3 A; 7 AN
7.58	A	10	0	10 AN	0	1 A; 9 AN	7	3 N
	B	10	0	10 AN	0	1 A; 9 AN	5	1 A; 1 N; 3 R

¹ Cumulative number of dead fish. Any mortalities were removed from the test chambers at each observation interval.

² Observations of surviving organisms: AN = appear normal; A = surfacing; R = lying on the bottom of the chamber; N = loss of equilibrium.



- 33 -

Table 9 (Continued)

Cumulative Mortality and Observations

Mean Measured Concentration (µg Permethrin a.i./L)	Rep.	No. Exposed	72 Hours		96 Hours		Cumulative Percent Mortality
			No. Dead ¹	Observations ²	No. Dead ¹	Observations ²	
Negative Control	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
Solvent Control	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
0.434	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
0.956	A	10	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	
1.48	A	10	0	10 AN	1	9 AN	5
	B	10	0	10 AN	0	10 AN	
3.12	A	10	8	2 A	10	--	95
	B	10	8	2 AN	9	1 A	
7.58	A	10	10	--	10	--	100
	B	10	10	--	10	--	

¹ Cumulative number of dead fish. Any mortalities were removed from the test chambers at each observation interval.

² Observations of surviving organisms: AN = appear normal; A = surfacing; -- = 100% mortality in the test chamber.

- 34 -

Table 10

LC50 Values Based on Mean Measured Test Concentrations

Time	LC50 (µg Permethrin a.i./L)	95% Confidence Interval (µg Permethrin a.i./L)	Statistical Method
24 Hours	> 7.58	-- ¹	NA ²
48 Hours	6.73	5.36 – 9.36	Probit Analysis
72 Hours	2.47	1.48 – 3.12	Nonlinear Interpolation ³
96 Hours	2.15	1.83 – 2.53	Probit Analysis

¹ 95% confidence limits could not be calculated with the mortality data obtained.
² NA = not applicable: <50% mortality precluded statistical calculation of an LC50 value.
³ The LC50 value was estimated using non-linear interpolation between 1.48 and 3.12 µg Permethrin/L.; the 95% confidence limits were determined by binomial probability.